



US005856298A

United States Patent [19]

Strickland

[11] Patent Number: 5,856,298

[45] Date of Patent: Jan. 5, 1999

[54] ERYTHROPOIETIN ISOFORMS

[75] Inventor: Thomas Wayne Strickland, Moorpark, Calif.

[73] Assignee: Amgen Inc., Thousand Oaks, Calif.

[21] Appl. No.: 334,882

[22] Filed: Nov. 3, 1994

Related U.S. Application Data

- [63] Continuation of Ser. No. 942,126, Sep. 8, 1992, abandoned, which is a continuation of Ser. No. 594,448, Oct. 12, 1990, abandoned, which is a continuation-in-part of Ser. No. 421,444, Oct. 13, 1989, abandoned.
- [51] Int. Cl.⁶ A61K 38/18; C07K 14/505; C07K 1/18; C07K 1/28
- [52] U.S. Cl. 514/8; 530/395; 530/397; 530/412; 530/416; 435/69.4; 204/182.9
- [58] Field of Search 530/395, 397, 530/416, 412; 514/8; 435/69.4; 204/182.9

[56] References Cited

U.S. PATENT DOCUMENTS

- 4,377,513 3/1983 Sugimoto et al. 530/395
 4,667,016 5/1987 Lai et al. 435/69.6
 4,703,008 10/1987 Lin 435/240.2
 4,801,540 1/1989 Hiatt et al. 435/172.3
 4,888,282 12/1989 Beremand et al. 435/172.3
 5,002,870 3/1991 Leavitt et al. 530/350
 5,451,662 9/1995 Naveh et al. 530/416

FOREIGN PATENT DOCUMENTS

- 267 678 5/1988 European Pat. Off. .
 0409113 1/1991 European Pat. Off. .

OTHER PUBLICATIONS

Fuhr et al., *Biochemical and Biophysical Research Communications* 98: 930-935, Feb. 27, 1981.

Viau et al., *Biochemical and Biophysical Research Communications* 117: 324-331, Nov. 30, 1983.

Havel et al., *Proc. Natl. Acad. Sci.* 77: 4349-4353, Jul. 1980.
 Welch et al., *Journal of Biological Chemistry* 259: 4501-4513, Apr. 1984.

Napier et al. "Isoelectric Focussing of Human Urinary Erythropoietin" *IRCS Med. SCI* 4:437 (1976).

Andreux et al. "Etude de l'erythropoietine. . ." *Annales Pharma. Francaises* 31(1):29-36, (1973).

Fuhr et al., *Biochem. & Biophys. Res. Comm.* 98: 930-935, Feb. 27, 1981.

Viau et al., *Biochem. & Biophys. Res. Comm.* 117: 324-331, Nov. 30, 1983.

Havel et al., *PNAS* 77: 4349-4353, Jul. 1980.

Krystal et al., *Blood* 67: 71-79, Jan. 1986.

Welch et al., *J. Biol. Chem.* 259: 4501-4513, Apr. 1984.

Dordal et al., *Endocrinology* 116: 2293-2299, 1985.

Sasaki et al., *J. Biol. Chem.* 262:12059-12076, Sep. 5, 1987.

Copsey et al., *Genetically Eng. Human Therap. Drugs*, pp. 25-26, 257, 1988.

Dube et al., *J. Biol. Chem.* 263:17516-17521, 25 Nov. 1988.

Goldwasser, "Erythropoietin & red cell differentiation", in *Control of Cellular Division and Devel: Part A*, pp. 487-494, 1981.

Lukowsky et al., "Studies on the role of Sialic Acid in The hysical and Biological Properties of Erythropoietin", *Can. J. Biochem.* 50:909-917, 1972.

Ashwell et al. *Methods Enzymol.* 50 , 287-288 (1978).

Bradford Anal. *Biochem.* 72 , 248-254 (1976).

Burnette et al. *Anal. Biochem.* 112 , 195-203 (1981).

Cotes et al. *Nature* 191 , 1065-1067 (Sep. 1961).

Davis et al. *Biochemistry* 26 , 2633-2638 (1987).

(List continued on next page.)

Primary Examiner—Stephen G. Walsh

Attorney, Agent, or Firm—Robert B. Winter; Steven M. Odre; Ron K. Levy

[57] ABSTRACT

Erythropoietin isoforms having a specific number of sialic acids per erythropoietin molecule are disclosed. Also disclosed are mixtures of such isoforms, pharmaceutical compositions containing such isoforms or mixtures thereof and methods of obtaining the erythropoietin isoforms.

31 Claims, 13 Drawing Sheets

in vivo U per mg Erythropoietin Polypeptide (Calculated from Radioimmunoassay)

